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Pandemic H1N1 2009 virus in Danish pigs: diagnosis and lack of surveillance

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In March-April 2009, a novel pandemic H1N1 virus (H1N1v) of likely swine origin emerged in the human population globally. The first case in pigs was reported from Canada in May 2009 and presently almost all countries with pig production have reported cases. The emergence of a new influenza subtype in swine with a genetic profile similar to older circulating strains implied a challenge for the veterinary diagnostic laboratories. We report the development, validation and implementation of a diagnostic strategy for specific diagnosis of H1N1v in pigs and the results of tests of pigs performed in Denmark.

Routinely, detection of swine influenza virus in clinical specimens is performed by real-time reverse transcriptase PCR assays (rRT-PCR) targeting the M and the NP genes. Alignment of the probe and primer sequences to available H1N1v gene sequences in GeneBank revealed that these assays most likely would recognize the H1N1v virus and this was further confirmed in the laboratory by test of samples from pvH1N1 infected humans. However, these assays could not discriminate between the typical circulating strains and the H1N1v subtype. For specific detection of the H1N1v subtype, an rRT-PCR assay targeting the HA gene developed at the Staten Serum Institute for diagnosis of H1N1v in humans was validated for use on pig specimens. *In silico* analysis showed that the probe and primers had 100% identity to published H1N1v strains and 80-95% identity to classical-swine H1N1 which do not circulate in Denmark. In contrast, there was only 60-70% identity to the subtypes circulating in Denmark (H1N1, H3N2, and avian-like H1N2) indicating that these subtypes would not be detected by this assay. The negative outcome of the test of 76 Danish swine influenza virus positive samples in the H1N1v assay confirmed that the assay was specific for H1N1v. Test of dilution series of cell culture adapted strains revealed a sensitivity of 1-2 TCID50/ML.

All influenza positive samples from swine submitted to NVI in 2009 (81 out of 299 submissions) have been tested for H1N1v with negative results. In 2010 (until the 24rd. of June) samples from 34 submissions have been tested and 5 herds were found positive for H1N1v (4 in January and 1 in June). The number of submissions for influenza diagnosis of swine have dropped significantly in 2010 compared to 2009 probably because the producers want to avoid the constraints put on the herd in case of a positive H1N1v result. In combination with the fact that Denmark does not have any formal surveillance program for swine influenza in place, we have currently no overview of the number of H1N1v positive swine in Denmark. However, the diagnosis of a positive herd in June 2010, outside the human influenza season, may indicate an ongoing swine to swine transmission of H1N1v in Denmark.

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